morphology producing ectopic "hinge points" that resemble the endogenous ventral midline hinge point — critical in bending, shaping and eventually closing the neural tube. Thus, we bring new insight into the mechanism underlying midbrain FP specification and show how *FOXA2* regulates both gene expression and cell shape.

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Program/Abstract # 422
A transition in *Sox2* gene regulation distinguishes the epiblastic and anterior neural plate states
Makiko Iwafuchi, Tatsuya Takemoto, Masanori Uchikawa, Yusuke Kamachi, Hisato Kondoh
Department of Developmental Biology, Osaka University, Suita, Osaka, Japan

The transcription factor gene *Sox2* is expressed in the epiblast and neural plate during the early embryonic stages in amniotes. Among the number of enhancers regulating *Sox2*, N-2 is most responsible for *Sox2* expression in the epiblast and anterior neural plate, as homozygous deletion of enhancer N-2 abrogates expression of *Sox2* in these tissue primordia.Here, the minimal essential sequence (core sequence) of enhancer N-2 was identified. Functional analysis of the regulatory elements was done using various mutated versions of the core sequences as performed by transfecting ES cells (as epiblast substitutes) and electroporating stage 4–5 chicken embryos (to assess neural plate activity). This analysis identified three POU factor binding sites (two overlapping) and an OTX binding site in the coresequence, as confirmed by EMSA. In ES cells with strong OCT3/4 expression, the N-2 core enhancer was primarily dependent on the activity of OCT3/4. In contrast, in the anterior neural plate, where OCT3/4 is down-regulated and OTX2 is strongly activated, the enhancer was dependent on OTX2 activity. In the *Otx2* knockout embryo, *Sox2* was expressed in the epiblastic stage but not in the anterior neural plate stage. Thus, the transition of *Sox2* regulation from OCT3/4-dependence to OTX2-dependence distinguishes the epiblastic and anterior neural plate states in early ectodermal lineages.

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Program/Abstract # 423
Detailed analysis of *zic1*, *zic2*, *zic3*, and *zic4* expression in trunk and hindbrain sections of early chick embryos
Ariel McMahon, Sara Muscarelli, Christa Merzdorf
Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA

The Zic family of transcription factors plays multiple roles in early development. *zic* genes are highly conserved, particularly in their zinc finger domains and in the regions immediately surrounding the zinc fingers. Using published sequences and the chicken genome as guides, we have generated in situ probes that are specific for the *zic1*, *zic2*, *zic3*, and *zic4* genes in chick. We have previously presented whole mount in situ comparisons for *zic1* and *zic2* with preliminary data on *zic3* and *zic4*. Now we have studied the expression of *zic3* and *zic4* in greater detail and present a detailed analysis of *zic1–4* expression in sections of stage 14/15 and stage 18/19 embryos. The *zic1–3* genes are expressed in overlapping patterns in the dorsal neural tube and in the dorsomedial portion of the somites, while *zic4* is expressed in the forebrain, but not in the hindbrain or trunk. *zic2* is the first *zic* gene expressed in the dorsal neural tube upon neural tube formation. *zic1* is the earliest *zic* gene expressed during somitogenesis. *zic3* is uniquely expressed in the presomitic mesoderm, although it is not expressed in newly formed somites. *zic2* is uniquely expressed throughout the neural tube of the tail tip and in the periotic mesoderm. Other differences will be discussed, comparisons with *zic* gene expression in other organisms will be made, and the expression patterns will be related to phenotypes resulting from aberrant *zic* gene expression.

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Program/Abstract # 424
Analysis of chicken paraxial mesoderm progenitor transcriptome using microarray technique
Bertrand Bénazéraf [a], Sachin Mathur [a], Karin Zueckert-Gaudenz [a], Gaye Hattem [a], Jayasinghe Sachintha [a], Tassy Olivier [a,b], Haug Jeff, Pourquié Olivier [a,b]
[a] *Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, Missouri 64110, USA*
[b] *Howard Hughes Medical Institute, Kansas City, MO 64110, USA*

The vertebrate body is subdivided along the antero-posterior axis into repeated segments. This pattern is established by the segmentation of the presomitic mesoderm (PSM) during embryogenesis. Cells that give rise to the PSM derive from the primitive streak and later from the tail bud. Because the segmentation process continues during antero-posterior (AP) axis elongation, the population of PSM cells must be continuously renewed. Different studies suggest the existence of paraxial mesoderm "stem cells" located first in the most anterior part of the primitive streak and then in the tail bud. While these cells appear to be of major importance in PSM production and in the set-up of the segmentation program, their cellular and molecular properties are not well understood. To better understand these properties, we use a DNA microarray approach in the chick embryo to identify genes specifically expressed in these precursors. Several candidate genes identified during this screen show specific expression in the zone of the paraxial progenitor stem cells by in situ hybridization. The function of these candidate genes will be tested in future work to know if whether or not they participate in the specific properties of paraxial mesoderm progenitors.

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Program/Abstract # 425
Identifying novel targets of Ptf1a using ChIP-on-chip technology
Sherri-Gae Scott, Steven D. Leach
[a] *Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[b] *Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Ptf1a is a bHLH transcription factor that is expressed in the progenitor cells of the dorsal bud at the onset of pancreas development. These progenitor cells eventually give rise to pancreatic ducts, endocrine and exocrine cells. As the pancreas develops, Ptf1a also functions to induce and maintain differentiation of the exocrine pancreas. In order to gain additional insight into the role of Ptf1a in mouse pancreas development, we intend to identify novel targets of this transcription factor and to investigate their role in pancreas development. We used chromatin immunoprecipitation (ChIP) *in vivo* to investigate the interaction between Ptf1a and genomic DNA in adult mouse pancreas, liver was utilized as a control tissue not expressing

Ptf1a. In addition to this we utilized DNA microarray (chip) technology to identify a set of genes enriched in the adult mouse pancreas that were not previously known to be associated with Ptf1a. As additional validating steps, we screened the genes for pancreatic expression using in situ hybridization, and evaluated promoter elements using luciferase assays, in order to further determine which genes directly interact and are regulated by Ptf1a. We believe that relevant target genes mediating the effects of Ptf1a on pancreatic development remain unknown. Using this ChIP-on-chip technology, our lab will be able to map stage specific changes in chromatin occupancy by Ptf1a in the developing mouse pancreas and identify novel targets of Ptf1a that have essential roles in pancreas development.

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Program/Abstract # 426
Modular patterning of structure and function of the striatum in the forebrain by retinoid receptor signaling
Fu-Chin Liu [a], Wen-Lin Liao [a], Pierre Chambon [b]
[a] *Institute of Neuroscience, National Yang-Ming University, Taipei, Taiwan*
[b] *Institut de Genetique et de Biologie Moleculaire et Cellulaire, College de France, Strasbourg, France*

Retinoid signaling plays a crucial role in patterning rhombomeres in the hindbrain and motor neurons in the spinal cord during development. A fundamentally interesting question is whether retinoids can pattern functional organization in the forebrain that generates high order of cognitive behavior. The striatum contains a compartmental structure of striosome (or 'patch') and intervening matrix. How this highly complex mosaic design is patterned by the genetic programs during development remains elusive. We report a developmental mechanism by which retinoid receptor signaling controls compartmental formation in the striatum. We analyzed RARβ$^{-/-}$ mutant mice and found a selective loss of striosomal compartmentalization in the rostral mutant striatum. The loss of RARβ signaling in the mutant mice resulted in reduction of cyclin E2 and Mash1, which led to defective neurogenesis of late-born striosomal cells. Importantly, during striatal neurogenesis, endogenous levels of retinoic acid were spatiotemporally regulated such that transduction of high levels of retinoic acid through RARβ selectively expanded the population of late-born striosomal progenitors, which evolved into a highly elaborate compartment in the rostral striatum. RARβ$^{-/-}$ mutant mice, which lacked such enlarged compartment, displayed complex alternations of dopamine agonist-induced stereotypic motor behavior. RARβ signaling thus plays a crucial role in setting up striatal compartments that may engage in neural circuits of psychomotor control.

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Program/Abstract # 427
Gbx2 and Fgf8 are sequentially required for formation of the mid-hindbrain compartment boundary
James Li, Abimbola Sunmonu, Qiuxia Guo
Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA

The mid-hindbrain boundary (MHB) is a cell-lineage restriction boundary and organizing center. However, the mechanism underlying MHB formation remains to be elucidated. In mouse embryos at E7.5, the presumptive MHB is demarcated by the common expression border of two homeobox genes *Otx2* and *Gbx2*. We have performed genetic inducible fate mapping using *Gbx2-CreER* KI mice. We show that cells expressing *Gbx2* at E7.5 strictly contribute to structures posterior to the MHB. By contrast, in *Gbx2*-null embryos, cells originated from the hindbrain abnormally contribute to the entire midbrain, while the cerebellum is missing. These results demonstrate that Gbx2 is a determinant of cerebellar progenitors and dictates lineage restriction at the MHB at E7.5. Furthermore, we provide evidence by chimera analysis that *Otx2*+ midbrain and *Gbx2*+ hindbrain precursors have different cell adhesive properties, suggesting that cell sorting based on differential affinities leads to initial MHB formation. Finally, we demonstrate that the MHB organizer *Fgf8*, which is expressed in a narrow domain immediately posterior to the MHB by E8.5, but not *Gbx2*, is essential for the refinement and maintenance of the lineage restriction at the MHB after E7.5. Our findings illustrate that the formation of the MHB is a stepwise process: differential expression of *Otx2* and *Gbx2* leads to segregation of midbrain and hindbrain precursors based on adhesive differences; the initial border is subsequently re-enforced by the induction of *Fgf8*, which further acts as an organizer to pattern the neighboring midbrain and hindbrain compartments.

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Program/Abstract # 428
***Six3*-promoted holoprosencephaly is caused by the absence of *Shh* expression in the rostral diencephalon ventral midline**
Xin Geng, Christina Speirs, Oleg Lagutin, Wei Liu, Lilianna Solnica-Krezel, Guillermo Oliver
[a] *Department of Genetics and Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105-2794, USA*
[b] *Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37235-1634, USA*

Holoprosencephaly (HPE), the most common forebrain malformation, is characterized by an incomplete separation of the cerebral hemispheres. Many genetic mutations, including those in *SHH* and *SIX3*, cause HPE. Using luciferase and zebrafish-based assays, we show that HPE-associated Six3-mutant proteins function ashypomorphs. Generated mouse models of Six3-promoted HPE revealed that *Six3* haploinsufficiency causes HPE in a strain-specific manner. Further, we demonstrate that *Shh* and *Six3* regulate each other in the rostral diencephalon ventral midline (RDVM). In mice displaying *Six3*-related HPE, this mutual regulation is disrupted, resulting in the loss of *Shh* and *Six3* in the RDVM, the loss of *Fgf8* and *Bmp4* signaling, abnormal apoptosis in the telencephalon, and ultimately HPE.

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Program/Abstract # 429
***Zic1* and *Zic4* are required for mammalian cerebellar patterning and growth**
Marissa C. Blank [a], Inessa Grinberg [b], Victor V. Chizhikov [b], Kathleen J. Millen [b]
[a] *Department of Molcular Genetics and Cell Biology, The University of Chicago, Chicago, IL, USA*
[b] *Department of Human Genetics, The University of Chicago, Chicago, IL, USA*